AMENDMENTS TO THE SPECIFICATION WITH MARKINGS TO SHOW CHANGES MADE

After the title, delete "Description".

Before paragraph **[0001]**, add the following:

--CROSS-REFERENCES TO RELATED APPLICATIONS

This application is the U.S. National Stage of International Application No. PCT/DE 2003/003179, filed September 9, 2003, which designated the United States and has been published as International Publication No. WO2004 028562 and which claims the priority of German Patent Application, Serial No. 10 244 863.9 filed September 23, 2002, pursuant to 35 U.S.C. 119(a)-(d).

BACKGROUND OF THE INVENTION--.

Before paragraph **[0024]**, add the heading --SUMMARY OF THE INVENTION--.

Before paragraph **[0081]**, add the heading --DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS--.

Page 37, after the heading "CLAIMS" and before the first claim add --What is claimed is:--.

Amend the following paragraphs:

--[0048] Generally, a connection between the strength of expression of an expression system and the resulting strength of the immune response is postulated, although numerous findings suggest that neither a linear correlation exists, nor that necessarily every treatment with expression vectors confers immunity in the desired strength (Wherry et al., Journal of Immunology 2002, 168 pp 4455-4461). For this reason, once the in-vitro expression results had been obtained, mice were immunised with peptide-linked expression vectors encoding the codon optimized and -signal optimized "env". The advantages of such

peptide linked constructs in eliciting an immune response are explained in detail in the disclosures of EP 0 941 318 B1 and DE 101 56 678 A1. In order to determine the immunologic importance of the p15 protein of "env" with regard to the provocation of an immune response, both sequences encoding "env" according to the invention were used (~~Seq. ID~~ SEQ ID NO: 7, 8, 9 and 10). The sera of immunized mice were assayed for specific antibodies against the FeLV viral protein "env" by means of Western blot. The antibody levels after the second immunization in week 4 clearly demonstrate that the synthetic constructs lead to a strong stimulation of antibody formation in-vivo, as well. In comparison, five of six mice of group 4 showed a strong immune response against the inventive antigen sequence, whereas the WT sequence (group 1) only led to a weak immune response in two of six animals (see FIG. 3).

[0054] SEQ ID NOS:	Sequence name-description
[0055] ~~Seq. ID~~ SEQ ID NO: 1	DNA sequence of the "env"-gene wild type
[0056] ~~Seq. ID~~ SEQ ID NO: 2	DNA sequence of the "gag" gene wild type
[0057] ~~Seq. ID~~ SEQ ID NO 3	Protein sequence of the "env"-gene wild type
[0058] ~~Seq. ID~~ SEQ ID NO: 4	Protein sequence of the "gag" gene wild type
[0059] ~~Seq. ID~~ SEQ ID NO: 5	DNA sequence of the mutated "gag" gene
[0060] ~~Seq. ID~~ SEQ ID NO: 6	Protein sequence of the mutated "gag" gene

[0061] ~~Seq. ID~~ SEQ ID NO: 7 DNA sequence of the mutated "env" gene (gp85). The gp70sequence is extended here by the sequence encoding the immunogenic p15 protein.

[0062] ~~Seq. ID~~ SEQ ID NO: 8 DNA sequence of the mutated "env" gene (gp70)

[0063] ~~Seq. ID~~ SEQ ID NO: 9 Protein sequence of the mutated "env" gene (gp85)

[0064] ~~Seq. ID~~ SEQ ID NO: 10 Protein sequence of the mutated "env" gene (gp70)

[0065] ~~Seq. ID~~ SEQ ID NO: 11 DNA sequence of the wild type of the "env" gene (gp70), derived from SEQ ID NO: 1 (NCBI database, Acc. No. M12500).

[0066] ~~Seq. ID~~ SEQ ID NO: 12 to ~~Seq. ID~~ SEQ ID NO: 40 sequences of the primers employed according to the following examples.

[0067] According to the invention, a DNA expression construct is provided for the expression of gene products of the feline leukosis virus (FeLV) in cat cells, consisting of a promoter sequence operable in Felidae and at least one nucleotide sequence that is related to a wild type nucleotide sequence encoding an original structural protein ("gag") and/or a membrane protein ("env") of FeLV, wherein said nucleotide sequence of FeLV is mutated and contains no open or hidden donor and/or acceptor sequences or a highly homologous but not identical part thereof. The proteins, which are highly homologous but not identical to the original membrane protein ("env") of FeLV, show a homology to the corresponding wild type of at least 98%. Preferred is an expression construct containing the sequences ~~Seq. ID~~ SEQ ID NO: 5, ~~Seq. ID~~ SEQ ID NO: 7 and/or ~~Seq. ID~~ 8 SEQ ID NO: 8.

[0070] According to the invention, also proteins are provided that are a protein highly homologous to the original structural protein (,,gag") of the Feline Leukosis virus (FeLV) (~~Seq. ID~~ SEQ ID NO: 6) or with the original membrane protein gp85 (,,env") of FeLV (~~Seq. ID~~ SEQ ID NO: 9) or with the original membrane protein gp70(,env") of FeLV (~~Seq. ID~~ SEQ ID NO: 10). These proteins in turn can be used for antibody production (monoclonal or polyclonal antibodies), which again in turn can be part of diagnostic kits for the diagnosis of infection of cats with feline leukosis virus.

[0113] FIG. 4: DNA sequence comparison of the wild type "gag" gene (~~Seq. ID~~

SEQ ID NO: 2) against the codon-optimized "gag" gene (~~Seq. ID~~ SEQ ID NO: 5). Similarity: 74.51%

[0114] FIG. 5: DNA sequence comparison of the wild type ,,env" (gp70 region from

~~Seq. ID~~ SEQ ID NO: 1) against the codon- and signal optimized ,,env" gene (gp70; ~~Seq. ID~~ SEQ ID NO: 8). Similarity: 75.75%

[0115] FIG. 6: DNA sequence comparison of the wild type ,,env" gene (~~Seq. ID~~ SEQ ID NO: 1) against the codon- and signal optimized ,,env" gene (gp85) (~~Seq. ID~~ SEQ ID NO: 7). Similarity: 80.25%

[0116] FIG. 7: Protein sequence comparison of the wild type "gag" protein (~~Seq. ID~~ SEQ ID NO: 4) against the protein sequence of the codon-optimized "gag" protein (~~Seq. ID~~ SEQ ID NO: 6). Similarity: 98.62%

[0117] FIG. 8: Protein sequence comparison of the wild type ,,env" protein (~~Seq. ID~~ SEQ ID NO: 3) against the protein sequence of the codon- and signal optimized ,,env" protein (gp70) (~~Seq. ID~~ SEQ ID NO: 10). Similarity: 98.75%

[0118] FIG. 9: Protein sequence comparison of the wild type ,,env" protein (~~Seq. ID~~ SEQ ID NO: 3) against the protein sequence of the codon- and signal optimized ,,env" protein (gp85) (~~Seq. ID~~ SEQ ID NO: 9). Similarity: 98.60%

[0120] The wild type sequences of the selected antigens, especially the "gag" gene, were obtained from the blood of infected cats. The DNA sequence of the "env" WT is given in ~~Seq. ID~~ SEQ ID NO: 1 (NCBI data base, Acc. No.: M12500), for the "gag" WT in ~~Seq. ID~~ SEQ ID NO: 2. The corresponding amino acid sequences are given in ~~Seq. ID~~ SEQ ID NO: 3 ("env") and ~~Seq. ID~~ SEQ ID NO: 4 ("gag").

[0122] In order to remove two Eco31I restriction sites, 3 PCR with the following mutation primers were performed:

gag-mut1-rneu (~~Seq. ID~~ SEQ ID NO: 12):
AATTAAGAGCTCCACGTCTCCCCCCGCTAACAGCAACTGGCG

gag-mut2-l (~~Seq. ID~~ SEQ ID NO: 13):
AATTAAGAGCTCCAGGTCTCCGGGGCTCCGCGGGGCTGCAAGACG

gag-mut3-r (~~Seq. ID~~ SEQ ID NO: 14):
AATTAAGAGCTCCACGTCTCCTTCCCTTTTGTTGTATATCTTTTCTGC

gag-mut4-l (~~Seq. ID~~ SEQ ID NO: 15):
AATTAAGAGCTCCAGGTCTCCGGAAACCCCAGAGGAAAGGGAAGAAAG

[0123] After ligation of the three sequences thus obtained, a PCR was performed with the primers:

Felvgag-l (~~Seq. ID~~ SEQ ID NO: 16):
CGGATAAGGTACCATGGGCCAAACTATAACTACC

Felvgag-r (~~Seq. ID~~ SEQ ID NO: 17):
TTCTCAGAGCTCTTAGAGGAGAGTGGAGTTGGCGGGT

[0124] Primer for "env" WT:

envl (~~Seq. ID~~. SEQ ID NO: 18):
CGGATAAGGTACCATGGCCAATCCTAGTCCACC envr

(~~Seq. ID~~. SEQ ID NO: 19):
AGTTCTCAGAGCTCTTAGGCTGTTTCAAGGAGGGCTT

[0129] Fragment 1:

left primer (~~Seq. ID~~ SEQ ID NO: 20):
ATATTGGATCCCATGGCCAACCCCTCCC

right primer (~~Seq. ID~~ SEQ ID NO: 21)
ATTATGGTCTCCTGCTGCTTCTTCCTGTCTGTGG

[0130] Fragment 2:

left primer (~~Seq. ID~~ SEQ ID NO: 22):
TAATAGGTCTCCAGCAGCAGACCTACCCCT

right primer (~~Seq. ID~~ SEQ ID NO: 23):
TAATAGGTCTCTGTGAACAGGGCAATGGGGTCA

[0131] Fragment 3:

left primer (~~Seq. ID~~ SEQ ID NO: 24):
TATTTGGTCTCTTCACAGTGTCCAGGCAGGTGTC

right primer (~~Seq. ID~~ SEQ ID NO: 25):
TATTAGGTCTCAGCTTGTGCTGGGGGGTGG

[0132] Fragment 4:

left primer (~~Seq. ID~~ SEQ ID NO: 26):
AATAAGGTCTCCAAGCTGACCATCTCTGAGGTGT

right primer (~~Seq. ID~~ SEQ ID NO: 27): ATTAAGAGCTCTCAGGCTGTTTCCAGC

[0133] total sequence:

left primer (~~Seq. ID~~ SEQ ID NO: 20):
ATATTGGATCCCATGGCCAACCCCTCCC

right primer (~~Seq. ID~~ SEQ ID NO: 27): ATTAAGAGCTCTCAGGCTGTTTCCAGC

[0135] Primer sequences for the complete signal sequence:

left primer (~~Seq. ID~~ SEQ ID NO: 28):
ATTGCCGGTACCATGGAGTCCCCCACCCACC

right primer (~~Seq. ID~~ SEQ ID NO: 29):
ATCAGAGGTCTCCCATGCCAATGTCAATGGTGAAC

[0139] Employed primer sequences:

left primer (~~Seq. ID~~ SEQ ID NO: 30): GATCTGGGTCTCCATGGCCAACCCCTC

right primer (~~Seq. ID~~ SEQ ID NO: 27): ATTAAGAGCTCTCAGGCTGTTTCCAGC

[0140] After digestion of the two PCR products with Eco31I, these were purified and ligated to each other. The ligation product was further processed in a PCR, in which a recognition sequence was generated for KpnI at the 5'-end and for SacI at the 3'-end.

[0141] Employed primer sequences:

left primer (~~Seq. ID~~ 28): ATTGCCGGTACCATGGAGTCCCCCACCCACC

right primer (~~Seq. ID~~ SEQ ID NO: 27):
ATTAAGAGCTCTCAGGCTGTTTCCAGC

[0146] Employed primer sequences:

left primer (~~Seq. ID~~ 31): AATTATGGTCTCGCAGTTCAGACAACTACAAATGGC

right primer (~~Seq. ID~~ SEQ ID NO: 32):
AATTATGAGCTCTCAGGGCCTGTCAGGGTC

[0147] 2. PCR:

[0148] The sequence of LeadFeLVenv was amplified. Thereby, a recognition sequence was generated at the 3'-end.

[0149] Employed primer sequences:

left primer (~~Seq. ID~~ SEQ ID NO: 33):
AATTATGGTACCATGGAGTCCCCCACCC

right primer (~~Seq. ID~~ SEQ ID NO: 34):
TATAATGGTCTCAACTGGGCTGTTTCCAGCAGGGC

[0150] After digestion of the two PCR products with Eco31I these were ligated to each other. The ligation product was processed in a PCR with the following primer sequences:

left primer (~~Seq. ID~~ SEQ ID NO: 33):
AATTATGGTACCATGGAGTCCCCCACCC

right primer (~~Seq. ID~~ SEQ ID NO: 32):

AATTATGAGCTCTCAGGGCCTGTCAGGGTC

[0159] Employed primer sequences:

left primer (~~Seq. ID~~ SEQ ID NO: 28):
5'-ATTGCCGGTACCATGGAGTCCCCCACCCACC

right primer (~~Seq. ID~~ SEQ ID NO: 35):
5'-ATATTAGGTCTCAGATCCGGGGGGGGGAGGG

[0162] Fragment 1:

left primer (~~Seq. ID~~ SEQ ID NO: 36):
ATATTGGTCTCAGGAGAGGGACAAGAAGAG
right primer (~~Seq. ID~~ SEQ ID NO: 37):
AATATGGTCTCTCAGCCTGCTGGCGATGGGGC

[0163] Fragment 2:

left primer (~~Seq. ID~~ SEQ ID NO: 38):
ATTATGGTCTCTGCACCTGAGGCTGTACAGGC

right primer (~~Seq. ID~~ SEQ ID NO: 39):
AATATGGTCTCGGTGCTCCCTGCCGGCGGGGGTGCA

[0164] Fragment 3:

left primer (~~Seq. ID~~ SEQ ID NO: 38):
ATTATGGTCTCTGCACCTGAGGCTGTACAGGC

right primer (~~Seq. ID~~ SEQ ID NO: 40):
AATATGGTCTCTCTCCTCCTGCCTCTGC

[0165] Primer for the complete fragment:
left primer (~~Seq. ID~~ SEQ ID NO: 36):
ATATTGGTCTCAGGAGAGGGACAAGAAGAG

right primer (~~Seq. ID~~ SEQ ID NO: 40):
AATATGGTCTCTCTCCTCCTGCCTCTGC

[0192] In group 2, 2 of 10 cats were free both of virus protein and of proviral DNA. This vaccine protection was based on the application of the inventive vaccine (~~Seq. ID~~ SEQ ID NO: 5).

[0193] In group 3, 40% of the animals could be protected by the inventive vaccine (~~Seq. ID~~ SEQ ID NO: 8) against infection with the FeL-Virus. This is a significant reduction of infected cats in comparison to group 1. –